PERFORMANCES OF POPULATIONS OF SEX REVERSED TILAPIA AND GENETICALLY MALE TILAPIA IN FINGERLING PONDS

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ABSTRACT

One of the most popular techniques in mass production of all or nearly all male tilapia population is through sex reversal. However, cross mating of YY super male with regular brood-stock is gaining popularity and has been practiced by aqua-culturists. This study was conducted to evaluate the performance of 2 populations of sex reversed tilapia (sex reversed with dietary application of 17-α methyl testosterone, SRT-1, and sex reversed with dietary administration of imidazole, SRT-2) and 1 population of genetically male tilapia (GMT) derived from YY super male until the end of fingerling rearing period. Mixed sex tilapia (MST) derived from normal mating was used as the control population. The results showed that the daily growth rates of SRT-1, SRT-2, GMT, and MST were 0.12, 0.11, 0.20, and 0.07 g/day, with survival rates were 57.83%, 64.67%, 39.67%, and 68.78%, respectively. The GMT has higher harvest yield than the other populations; SRT-1 (+15.73%), SRT-2 (+6.37%), and MST (+20.82%).

KEYWORDS: Growth performance, sex reversal, 17-α methyl testosterone, aromatase inhibitor, genetically male tilapia, Nile tilapia

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is one of indigenous fishes from Africa and one of the most popular species in aquaculture industry (Popma & Lovshin, 1995). In Indonesia, tilapia culture was started in 1969. Now, Nile tilapia is one of the top ten species in aquaculture (Dirjen Perikanan Budidaya, 2005). Biologically, tilapia has a sexual dimorphism, where the male grows faster than the female (Popma & Masser, 1999). Due to this characteristic, monosex tilapia culture especially male monosex culture will give higher harvest yield than mixed-sex population (Rakocy & McGinty, 1989; Mair *et al*., 1995; Tave, 1996; Champan, 2000; Dunham, 2004; Gustiano *et al*., 2006).

Sex reversal is one of several techniques for mass production of all male population in fish, including Nile tilapia culture. The most popular technique in sex reversal is through direct hormonal application with androgen hormone, such as masterolone or 17-α methyl testosterone. Alternatively, sex reversal can be performed by inhibiting aromatase enzyme, for blocking synthesis of estrogen from androgen which is catalyzed by this enzyme during sexual differentiation period. Another technique for mass production of all male population is through the production of the novel YY male genotypes. If a YY super-male crosses with normal female that has XX genotypes, the result would be all or nearly all male progeny which have XY genotypes (genetically male tilapia, GMT). The initiation and development programs of Indonesian YY super-male are being carried out at the Institute for Aquaculture Development, Sukabumi. This program had successfully launched the first

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new variety of YY super-male which is called 'GESIT' or Genetically Super-male of Indonesian Tilapia in 2007. Procedure of Indonesian YY super-male program can be seen in the Standard Procedure Operational #5, 2004 of National Brood-stock Centre for Tilapia (Anonymous, 2004).

The objective of this study was to study the performance of 2 populations of sex reversed tilapia through direct hormonal application of 17-α methyl testosterone and dietary administration of imidazole as the aromatase inhibitor, and then compare it with genetically male tilapia derived from YY super-male, in fingerling ponds. Mixed sex tilapia derived from normal mating was used as the control population.

MATERIALS AND METHODS

The materials for sex reversal treatment and control were Nile tilapia fry of 7 days after hatching weighted 0.007–0.009 g/individual fish. Nirwana tilapia strain originated from the Institute for Aquaculture Development in Wanayasa was used as brood stock. The brood-stocks were mated and spawned in earthen ponds at ratio of 1:2 for male and female. GMT population was obtained by crossing YY super-male originated from the Institute for Aquaculture Development in Sukabumi with female of Nirwana variety. Mating design used was the same process as in normal mating. Eggs were collected after spawning process in the ponds 2 weeks after male and female brood stocks pooled. Incubating and hatching of eggs were conducted in hatching trays and they completely hatched after 3–5 days. All fry were reared in fiberglass batch with equivalent densities.

Experiment methods

Experimental design used was a completely randomized design (CRD) with single factor, i.e. (1) sex reversed tilapias through direct hormonal application of 17-α methyl testosterone (SRT-1) with the dosage of 60 mg/kg of feed (Anonymous, 2004; Bowker *et al.*, 2007), (2) sex reversed tilapias through dietary administration of aromatase inhibitor (SRT-2) with the dosage of 25 mg imidazole/kg of feed (Ariyanto *et al.*, 2009), (3) genetically male tilapias (GMT), derived from YY super-male which was mated with normal female and (4) mixed sex tilapia derived from normal matting of tilapia (MST). All treatments were done in 3 replications.

Friy rearing were conducted in 12 aquaria sized 60 cm x 40 cm x 40 cm. The density of fish was 5 fishes/liter, equivalent to 300 fishes in each aquarium. Fish were fed daily with artificial feed (40% crude protein) at satiation, 4–5 times each day. Feed containing androgenic hormone and aromatase inhibitor was given to the fishes for 4 weeks. Siphoning and 30%–50% water exchange were done on each aquarium every day.

At the end of the 4th week, all fish from each aquarium with average weight of 0.11–0.14 g/individual fish were transferred to 12 unit happas sized 2 m x 2 m x 1 m. All happas were placed in a 400 m^2 earthen pond. The fishes were fed with artificial pellet feed (30% crude protein) at satiation for 8 weeks. Feed was given 3 times/day, at 09.00 AM, 01.00 PM, and 05.00 PM. Samplings for average body weight and length of fish was done every 4 weeks. Number of sample for each happa was 30 fishes. At the end of this experiment, all fish were harvested and weighted to determine the survival rate and total weight (biomass).

Data analysis

The data collected from all treatments and replications were male proportion, average body weight and length, survival rate, and total weight (biomass) at harvest. Means of daily growth rate were calculated by:

$(Wt\text{-}W0).t^{-1}$ where: Wt is average body weight at harvest (12 weeks), W0 is average body weight at t-0, and t is the time of fish rearing (12 weeks 84 days)

As supporting data, water quality parameters, i.e. temperature, pH, dissolved oxygen, nitrite and ammonia in each aquarium and happa were observed every 2 weeks. All data were analyzed using two ways ANOVA on treatment and replicate means. Duncan's multiple range test was used to determine significance of difference ($P < 0.05$) between treatment means (Steel & Torrie, 1993). Data of the water quality parameters were analyzed using descriptive statistic.

RESULT AND DISCUSSION

Male proportion, average weight and length, survival rate and total weight of each population at the end of fingerling rearing

Table 1. Male proportion (%), average body weight (g), average body length (cm), survival rate (%) and total weight (g) of fishes at end of fingerling rearing period

Populations	Male proportion	Average		Survival rate	Total weight
		Weight	Length		
SRT-1	81.85±7.69^{a}	10.37±4.12^{ab}	7.98±0.04^{ab}	57.83±26.16^{ab}	1,637.30±99.50^{a}
SRT-2	81.25±15.99^{a}	9.44±0.80^{a}	8.88±0.18^{a}	64.67±9.90^{a}	1,819.12±124.47^{a}
GMT	68.61±13.78^{a}	16.56±1.81^{c}	9.82±1.54^{a}	39.67±10.37^{b}	1,942.94±300.19^{b}
MST	58.54±3.31^{b}	6.18±0.17^{b}	7.80±0.57^{b}	68.78±24.64^{ab}	1,538.39±7.37^{c}

Note: The numbers followed by the same superscripts in the same column are not significant different (P>0.05)

period in happas which were placed in a 400 m^2 earthen pond are presented in Table 1.

Sex reversal and male proportion

In general, direct hormonal application of 17-α methyl testosterone and dietary administration of imidazole as aromatase inhibitor increased the male proportion of tilapia population. GMT, SRT-1, and SRT-2 populations have higher male proportion than MST population as the control. Statistical analysis showed that the male proportion of SRT-1, SRT-2, and GMT populations were significantly different from MST population (P<0.05). Both of SRT-1 and SRT-2 populations were not significantly different from GMT population (P>0.05). This results indicated that direct application of 17-α methyl testosterone and dietary administration of imidazole in this study were effective to increase the male proportion in tilapia population. On the other hand, using the GESIT variety as the YY super-male was effective to get nearly all male in tilapia population, although 100% male was not reachable.

One of the main parameters in successful sex reversal techniques application is the sex proportion of population. In this experiment, sex reversal in tilapia is the main objective to produce all or nearly all male population. In comparison with control population, both direct hormonal applications of 17-α methyl testosterone and dietary administration of imidazole increased male proportion within these populations. The application of 17-α methyl testosterone hormone which was aimed to increase the level of testosterone in fish body, successfully improved male proportion up to 81.85%, equivalent with 39.82% higher than control population (58.54%). This result is in line with several early studies on direct application of 17-α methyl testosterone hormone for sex reversal in fish. Gustiano (1992) showed that direct application of 15 mg 17-α methyl testosterone hormone per kg of feed increased the male proportion of Nile tilapia up to 79%. Furthermore, Mantau *et al.* (2001), Guerrero III & Guerrero (2004) and Bowker *et al.* (2007) showed that application of synthetic androgen at dosage of 15, 50, and 40 mg 17-α methyl testosterone hormone per kg of feed also increased the male proportion up to 93%, 96%, and 95%, respectively. Lower male proportion in this study than that of earlier studies may be caused by paradoxical effect phenomenon. Irfan (1996) reported that high concentration of methyl testosterone hormone (50 mg/200 g of feed) in *O. niloticus* produced 83.54% of male and 16.46% female. The other study conducted by Haniffa *et al.* (2004) reported that immersion of eggs of *Heteropneustes fossilis* catfish in high concentration (400 µg/L) of methyl testosterone produced 30% of male and 70% of female.

Dietary administration of imidazole was effective in inhibiting the secretion of aromatase enzyme that catalyzes estrogen synthesis during sexual differentiation period. The dosage of dietary administration of imidazole in this experiment (25 mg/kg of feed) successfully improved the male proportion of tilapia population up to 81.25%, equivalent with 38.79% higher than control population (58.54%). Kwon *et al.* (2000) reported that dietary administration of fadrozole with dosage 500 mg/kg of feed at 13 days after hatching of Nile tilapia larvae for 5 days improved the proportion of male up to 80%. Furthermore, Afonso *et al.* (2001) showed that dietary administration of fadrozole for 21 days with the dosage of 75 mg/kg of feed at 9 days after hatching of nile tilapia larvae improved the male proportion nearly 100%. In Indonesia, research on sex reversal in nile tilapia trough inhibition of aromatase enzyme secretion role was done by Nurlaela (2002), Astutik (2004), and Barmudi (2005). These studies showed that imidazole treatment as aromatase inhibitor trough dipping method at embryonic and fry phase of red tilapia improved the male proportion up to 82.22%, 57.97%, and 73.09%, respectively.

Based on the previous discussion on sex reversal techniques, crossing of YY super-male with normal female improved male proportion about 17.20% from control population derived from normal mating. The super male which has YY genotype crossed with normal female which has XX genotype, should get all male genotype (XY) progeny. In this experiment, the male phenotype proportion was obtained about 68.61%. All male phenotype could not reach 100% in this experiment due to several reasons. Firstly, sex is genetically determined by chromosome (Yatim, 1986) and occurs during fertilization period (Maty, 1985). Yamamoto (1969) stated that if male factor is more dominant than female, the zygote will develop and become a male. But, differentiation of sex is a developmental process and very labile until the definitive gonad tissue occurred. Zairin (2002) explained that sex differentiation process consists of several phenomena where sex genotype could be expressed as phenotype sex. Although early determination of individual sex of fish is determined by itself genome, but the expression to the sex phenotype is affected by surrounding environment like biochemical factors (Chan & Yeung, 1983). Dunham (1990) explained that although sex genotype was determined during fertilization, determination of phenotype sex occurs during the developmental stage of fish larvae. If the developmental time of fish is intervened by environmental factors or is supplemented by other materials, reversed determination of sex phenotype can occur (Zairin, 2002). Secondly, among several physical factors except the biochemical factors, temperature of the environment strongly affects sex differentiation process. The constant high temperature is inclined toward the male sex phenotype, and the fluctuating low temperature determines sex phenotype of female (Baras *et al*., 2000 *in* Devlin & Nagahama, 2002). Baroiller *et al.* (1999) showed that higher temperature affected sex ratio in *O. niloticus.* Rearing fish in temperature of 33°C–37°C improved male ratio up to 60%–100%. Result analysis of water temperature during fish rearing period in aquaria ranged from 26.10°C to 26.60°C. The low temperature is probably one of the main physical factors that causes male ratio of this population can not reach 100%.

Growth

The pattern of growth of each population during the 12 weeks trials is described in Figure 1. The growth rate of all populations in aquaria was not significantly different (P>0.05) (Figure 1), but there was a significant difference in fingerling pond (P<0.05). At the end of fingerling rearing period in the pond, both SRT populations and GMT population had higher mean body weight than MST population. GMT population had the highest individual mean body weight followed by SRT-1 and SRT-2. Statistic analysis showed that means of individual body weight of SRT-1, SRT-2, and GMT population were significantly different from MST population (P<0.05).

Means of daily growth rate of SRT-1, SRT-2, GMT, and MST were 0.12, 0.11, 0.20, and 0.07 g/day, respectively. At the end of fingerling rearing period, the total weight at harvest (SRT-1 and SRT-2) was significant difference from GMT and MST populations. It is equivalent to 4,093,250, 4,547,800; 4,857,350; and 3,845,975 kg/ha/cycles. The significant difference of male proportion between SRT-1, SRT-2, and GMT from MST population was suspected as the main caused factor. The male fish that had faster growth rate than female in SRT-1, SRT-2, and GMT population (68.61%–81.85%) causes higher the total weight of these populations than MST population. Except it is cause by sexual dimorphism, high growth rate of GMT population is possible due to the low survival of this population (39.67%). Because of less number of individual fish in GMT population, competition level among the fish for several activities such as feed, space and oxygen consumption was low. Thus, this low competition level had lowered the energy requirements on that aspect. Decreasing of energy requirements for feeding, moving and breathing had allowed fish to transfer the energy in body mass building. This phenomenon may be the case why the means

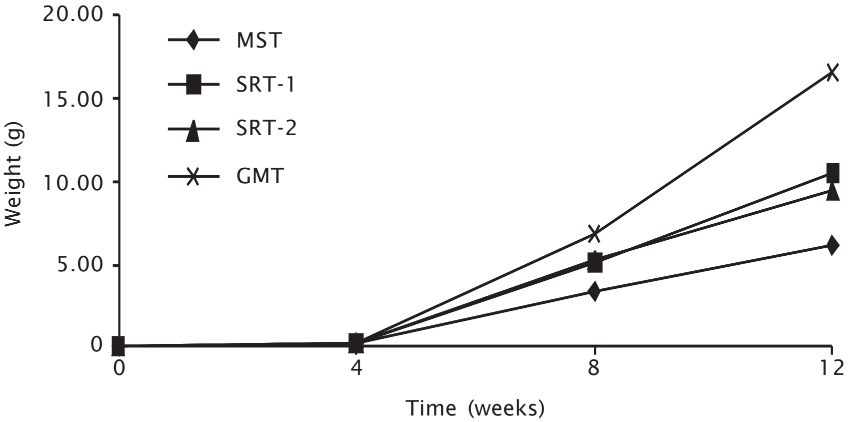

Figure 1. The pattern of growth rate in each population of Nile tilapia during fingerling rearing period

of body weight and the total weight of GMT population at harvest were higher (16.56 g and 1942.94 g) than that of the others.

Survival rate and water quality

The pattern of survival rate of each population during the 12 weeks trials is described in Figure 2. The mortality rate of all populations in aquarium during the rearing period was relatively higher than that of in fingerling ponds. At the end of fingerling rearing period, GMT population had the lowest survival rate, about 39.67% (Table 1). Although it had the lowest survival rate, the GMT population had the highest mean body weight and total weight at harvest.

Variation of water quality parameters for aquarium rearing and fingerling rearing periods are presented in Table 2.

Water quality in fingerling ponds was suitable and optimum for fish culture. However, water quality was poor for larvae and fry, especially for nitrite and ammonia levels, i.e. 0.03–0.49 mg/L and 0.00–0.33 mg/L in the aquaria.

The survival rates of all populations were generally low to medium, ranging from 39.67% for GMT to 68.78% for MST. Result from previous research on sex reversal in finfish and crustacean showed that there was no relationship between treatment and mortality

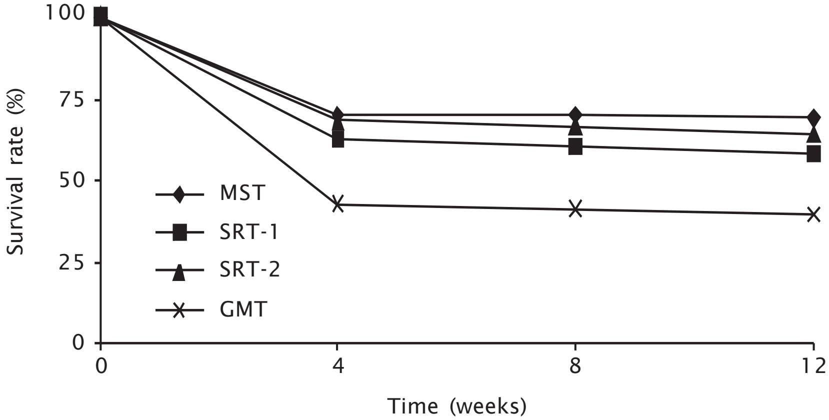

Figure 2. Pattern of survival rate in each population of Nile tilapia during fingerling rearing period

Table 2. Water quality in aquaria and fingerling ponds

Parameters	Aquaria	Ponds
Temperature (°C)	26.10–26.60	29.70–30.00
pH	7.91–8.22	7.73–7.80
Dissolved oxygen (mg/L)	6.36–3.64	5.48–3.40
Nitrite (mg/L)	0.03–0.49	0.04–0.07
Ammonia (mg/L)	0.00–0.33	0.10–0.26

rate. Kwon *et al.* (2000) explained that there was no statistical relationship between aromatase inhibitor treatments for Nile tilapia with mortality of fish. This statement was supported by Mazida (2002) for *Poecilia reticulate* and Muthalib (2004) for *Macrobrachium rosenbergii*, and Nurlaela (2002), Astutik (2004), and Barmudi (2005) for red tilapia. The first factor that contributed to the low of survival rate in this research was low dissolved oxygen because the recirculation system was not provided on the dense population (300 fishes/aquarium). Although the water exchange and siphoning for faecal and feed wastes were done every day to maintain water quality condition, the waste materials were not completely removed and thus degrading water quality condition in the aquarium. Poor water quality, such as high concentration of nitrite ($N\text{-}NO_2^-$) and ammonia ($N\text{-}NH_3$) was suspected as the second factor which contributed to higher mortality. Analysis of water quality parameters showed that the concentration of dissolved oxygen decreased from 6.36 to 3.64 mg/L, and both concentrations of nitrite and ammonia increased from 0.03 to 0.49 and from 0.0023 to 0.33 mg/L, respectively. Ammonia and nitrite are produced from dissolved waste materials from fish metabolism and feed. At high concentration, both ammonia and nitrite are hazardous for aquatic animal including fishes. Boyd (1990) suggested that nitrite and ammonia in the medium for fish culture should be less than 0.4 and 0.5 mg/L respectively. Concentration of nitrite in aquarium was relatively high starting in the middle to the end of this study and had caused high mortality of treated fish. Although the concentration of ammonia was still below the maximum threshold, 0.33 mg/L, but it was in the critical amount especially for larvae and fry stages of fish. The fish with mean body weight under 1 g/fish were less tolerant to poor water quality such as temperature, salinity, nitrite, and ammonia. This condition caused the survival rate in aquarium rearing period was low. But in happas, where water quality was relatively better than in the aquarium, the survival rate in this period was high.

High performance of fish especially in growth and survival rate is required in aquaculture. High growth performance of fingerling fish in this study is expected to express in the grow-out period.

CONCLUSIONS

1. Both of sex reversal and GMT populations produced higher male proportion than control population.
2. GMT population has the highest growth rate and harvest.

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REFFERENCES

Afonso, L.O.B., Wassermann, G.J., & de Oliveira, R.T. 2001. Sex reversal in Nile tilapia (*Oreochromis niloticus*) using a nonsteroidal aromatase inhibitor. *J. Exp. Zool.*, 290: 177–181.

Anonymous. 2004. Standar Operasional Prosedur (SPO) No. 05 Tahun 2004. Sex reversal pada ikan nila. PPINN-Pusat Produksi Induk Nila Nasional.

Ariyanto, D, Listiyowati, N., & Himawan, Y. 2009. Aromatase *inhibitor*, bahan alternatif pengganti hormon methyltestoterone pada *sex reversal* ikan nila. *Forum Inovasi Teknologi Akuakultur*. Surabaya, 9–13 Juli 2009. Pusat Riset Perikanan Budidaya. 12 hlm.

Astutik, I.O. 2004. ***Sex reversal** pada ikan nila merah melalui perendaman larva dengan aromatase **inhibitor***. Skripsi. Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor. 33 hlm.

Barmudi, I. 2005. *Efektivitas aromatase **inhibitor** terhadap **sex reversal** ikan nila merah dalam suhu media 33ºC*. Skripsi. Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor. 27 hlm.

Baroiller, J.F., Guiguen, Y., & Fostier, A. 1999. Endocrine and environmental aspects of sex differentiation in fish. *Cell Mol Life Sci*., 55: 910–931.

Bowker, J., Bowman, M.P., Carti, D., & Dotson, M. 2007. Histological determination of Tilapia gender following treatment with 17 á-methyl testosterone. Aquaculture America 2007. Therapeutic drug research session, February 2007. San Antonio TX. 21 pp.

Boyd, C.E. 1990. Water Quality in Pond for Aquaculture. Auburn University, Alabama. 482 pp.

Chapman, F.A. 2000. Culture of Hybrid Tilapia: A Reference Profile. SIR. 1050. Univ. of Florida. 5 pp.

Chan S. T. H. & Yeung, W.B. 1983. Sex control and sex reversal in fish under natural condition. *In*: Hoar W. S. and D. J. Randall. (Eds.). Fish Physiology. New York. Academic Press. IXB: 171–222.

Devlin, R.H. & Nagahama, Y. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture*, 208: 191–364.

Dirjen Perikanan Budidaya, DKP. 2005. Evaluasi dan target produksi perikanan budidaya Indonesia tahun 2005–2009. Rakornas Dept. Kelautan dan Perikanan. Jakarta.

Dunham, R.A. 1990. Production and use of monosex a sterill fishes in aquaculture. *Aquaculture Sci*., 2: 1–17.

Dunham, R.A. 2004. Aquaculture and Fisheries Biotechnology: Genetic Approaches. CABI Publishing. 384 pp.

Guerrero III, R.D. & Guerrero, L.A. 2004. Effects of androstenedione and methyl testosterone on *Oreochromis niloticus* fry treated for sex reversal in outdoor net enclosures. www.nraes.org/publications. www.aq.arizona.edu.

Gustiano, R. 1992. Penggunaan hormon dalam pakan pada pembentukan ikan nila jantan. Warta Penelitian dan Pengembangan Pertanian. XIV(5): 15–16.

Gustiano, R. 2006. Perbaikan mutu genetik ikan nila. Balai Riset Perikanan Budidaya Air Tawar, Bogor. 6 hlm.

Haniffa, M.A., Sridhar, S., & Nagarajan, M. 2004. Hormonal manipulation of sex in stinging catfish *Heteropneustes fossilis* (Bloch). Curr. Sci. 86(7): 1012–1017.

Irfan, M. 1996. Penggunaan hormon testosterone dengan dosis berbeda terhadap pembentukan individu jantan, mortalitas dan pertambahan berat benih ikan nila (*Oreochromis niloticus*). Fakultas Perikanan dan Ilmu Kelautan, Universitas Sam Ratulangi, Manado.

Kwon, J.Y., Hashpanah, V., Hartudo, L.M., McAndrew, B., & Penman, D. 2000. Masculinization of genetic female Nile Tilapia by dietary administration of an Aromatase inhibitor during sexual differentiation. *J. of Exp. Zool*., 287: 46–53.

Mair, G.C., Abucay, J.S., Beardmore, J.A., & Skibinski, D.O.F. 1995. Growth performance trials of genetically male tilapia (GMT) derived from YY-males in *Oreochromis niloticus* L. : On station comparisons with mixed sex and reversed male populations. *J. Aqu*., 137: 313–322.

Mantau, Z., Supit, A., Sudarty, Rawung, J.B.M., Buchari, U., Oroh, L., Sumampow, J., & Mamentu, A. 2001. Penelitian adaptif pembenihan ikan mas dan maskulinisasi ikan nila di Sulawesi Utara. Laporan Hasil Penelitian. Instalasi Penelitian dan Pengkajian Teknologi Pertanian Kalasey, Sulawesi Utara.

Maty, A.J. 1985. Fish Endocrinology. England. 265 pp.

Mazida, A.N. 2002. Pengaruh aromatase *inhibitor* terhadap nisbah kelamin ikan gupi (*Poecilia reticulata* Peters). [Skripsi]. Bogor: Fakulas Perikanan dan Ilmu Kelautan, Institut Pertanian Bogor.

Muthalib, A. 2004. *Pengaruh lama pemberian aromatase inhibitor melalui pakan terhadap nisbah kelamin udang galah (**Macrobrachium rosenbergii** de Man)*. [Skripsi]. Bogor: Fakultas Perikanan dan Ilmu Kelautan, Institut Pertanian Bogor.

Nurlaela. 2002. *Pengaruh dosis aromatase inhibitor pada perendaman embrio terhadap nisbah kelamin ikan nila merah (**Oreochromis niloticus**)*. Skripsi. Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor.

Popma, T.J. & Lovshin, L.L. 1995. Worldwide prospects for commercial production of tilapia. International Center for Aquaculture and Aquatic Environments. Department of Fisheries and Allied Aquacultures. Auburn University, Alabama 36849. 42 pp.

Popma, T.J. & Masser, M. 1999. Tilapia : Life history and biology. SRAC Publ. No. 283. 4 pp.

Rakocy. J.E. & McGinty, A.S. 1989. Pond culture of tilapia. SRAC Publ. No. 280. 4 pp.

Steel, R.G. & Torrie, J.H. 1993. Prinsip dan Prosedur Statistik. PT Gramedia Pustaka. Jakarta.

Tave, D. 1996. Selective breeding programmes for medium sized fish farms. FAO Fish. Tech. Paper 352. 122 pp.

Yamamoto, T. 1969. Sex differentiation. *In*: Hoar, W. S. and D. J. Randall. (Eds). Fish Physiology. Vol. III. Academic Press. New York. 117–158.

Yatim, W. 1986. Genetik. Tarsito, Bandung. 397 hlm.

Zairin, M. J. 2002. *Sex reversal*: memproduksi benih ikan jantan atau betina. Penebar Swadaya, Jakarta. 96 hlm.